Disk Electrophoretic Analysis of Growth Hormone and Prolactin in the Anterior Pituitary of the Golden Hamster

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Disk Electrophoretic Analysis of Growth Hormone and Prolactin in the Anterior Pituitary of the Golden Hamster

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Summary

The present experiment was carried out in order to determine the growth hormone and the prolactin in the anterior pituitary of the golden hamster. The analytical electrophoresis was performed by the disk-electrophoretic method.

Four intensely stained components were recognized in the homogenates of the pituitary glands. The most slowly migrating band was identified as growth hormone and the faint yellow-brown color band, just in front of the growth hormone, had the same electrophoretic mobility as a sample of hemoglobin of the hamster. The most rapidly moving band was identified as prolactin.

The band designated as prolactin and growth hormone in the electrophoretic elute were tested for their hormonal activity by the pigeon crop sac assay and the tibial method. The growth hormone of hamster was found to be four times as active as bovine growth hormone (NIH–B12). The minimum effective dose of hamster prolactin measured on the pigeon crop sac assay might be around 25 μg protein equivalent.

Introduction

The disk-electrophoretic method of Ornstein (1) and Davis (2) has been employed for analysis of pituitary hormones of several species (3–9), from different view points. But, it has not been used for analysis of pituitary hormones of the golden hamster (Mesocricetus auratus).

The present experiments were carried out in order to identify the growth hormone and the prolactin of the anterior pituitary of the golden hamster and to estimate the activity of these hormones.

Materials and Methods

Electrophoresis. Analytical electrophoresis on 7.5 per cent polyacrylamide at pH 9.5 was performed by the method of Nagai (10). Electrophoretic runs were made in the regular 0.5×7 cm columns for 2 hr at 300 V and 3 mA per column.

Bromphenol blue was used to mark the buffer front. Proteinaceous components were stained with 1 per cent Amido Black 10 B in 7 per cent acetic acid for 1 hr and destained electrophoretically at room temperature.

Preparation of pituitary gland homogenates. The experimental animal was killed with chloroform and the pituitary gland was removed immediately. The anterior lobe of the pituitary gland was dissected from the posterior lobe and was rinsed in saline in order to eliminate adhering blood. The anterior lobe was ground in a small glass hand-homogenizer with total capacity of 0.5 ml using 0.01 ml of 0.05 M phosphate buffer, pH 7.5, for each mg of tissue, and mixed with an equal volume of the upper gel used for electrophoresis. The homogenizer was rinsed three times with a few drops of upper gel solution and this solution was combined with the sample gel solution. The homogenate was transfered quantitatively with additional upper gel to the electrophoretic column.

Identification of albumin and hemoglobin. The albumin was salted out from the serum of hamsters by the sodium sulfate system method of Kekwick (11, 12) and hemoglobin was extracted by the method of Kovach et al. (13). These preparations were added to the upper gels with the suitable concentrations and their mobilities were examined electrophoretically.

Elution of growth hormone and prolactin and assay procedures. The growth hormone and the prolactin of the hamster were electrophoretically eluted by a modification of the method of Lewis and Clark (14). The proteins obtained were assayed in the rat for growth hormone by the tibial method of Ando et al. (15). The assay for prolactin was performed as described by Lyons and Page (16). The concentration of protein in samples to be assayed was determined by the method of Lowery et al. (17), using bovine serum albumin as the primary standard.

Results

Electrophoretic patterns of glands of hamsters. Fig. 1 shows the electrophoretic patterns (pH 9.5) obtained with homogenates of anterior lobe of pituitary glands from male and female hamsters. The main proteinaceous components were stained with Amido Black 10 B and four bands were recognized. The band B was identified as serum albumin. The component labeled as albumin migrated with the same mobility as serum albumin of the hamster. The rather faint band C just in front of the band D had the same electrophoretic mobility as a sample of hemoglobin of the hamster. This band possessed a faint yellow-brown color which was visible on unstained columns. The bands corresponding to albumin and hemoglobin were always more intense when the pituitary glands were not rinsed before analytical analysis. The most slowly moving band D was identified as growth hormone and the most rapidly moving band A was identified as prolactin.

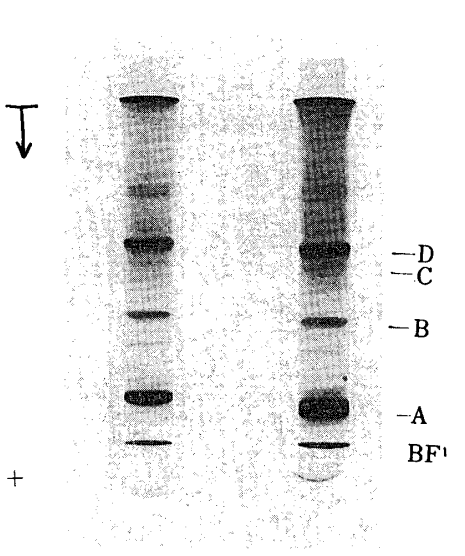

Fig. 1

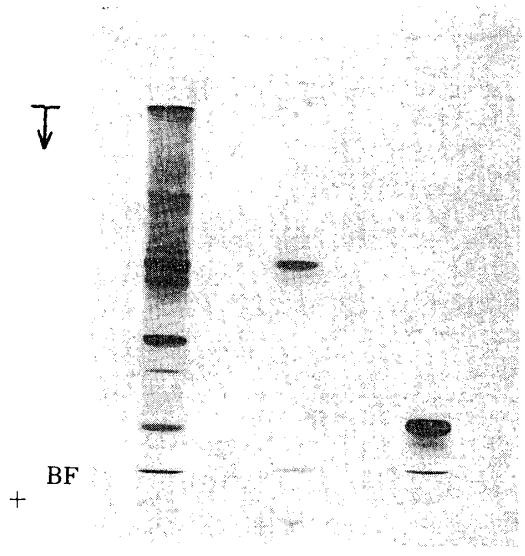

Fig. 2

FIG. 1. Electrophoretic patterns (pH 9.5) of the anterior pituitary gland of an adult hamsters.
A: Prolactin, B: Albumin, C: Hemoglobin, D: Growth hormone, BF: Buffer front.

FIG. 2. Electrophoretic patterns (pH 9.5) of a homogenate of anterior pituitary gland of hamster (A), growth hormone band (B) and prolactin band (C), each was eluted electrophoretically.
BF: Buffer front.

Both bands were more deeply stained in the female than in the male.

Fig. 2 shows the electrophoretic patterns of anterior pituitary homogenate, extracted growth hormone and prolactin. The major bands obtained with pituitary homogenate showed the same mobility as that of the extracted growth hormone and prolactin.

Hormonal activity of major bands. In order to detrmine the kind of hormone in the principal bands, the material was electrophoretically eluted from the gel and assayed for hormonal activity. The activity of the eluted growth hormone was compared with bovine growth hormone (NIH-B12). Hypophysectomized rats of Wistar strain at 6 weeks of age were separated into five groups consisting of five animals each. One group was injected with physiological saline as a control. Two groups were given bovine growth hormone 25 μg/day and 50 μg/day subcutaneously for 4 days. The other groups were injected with eluted hamster growth hormone 6.25 μg/day and 12.5 μg/day for 4 days. All groups were killed at 24 hr after the last injection, and the tibial cartilage width was measured.

The results are illustrated in Table 1. The hamster growth hormone was more active than the bovine growth hormone, and the former had four times the potency of the latter. Also, the body weight gain of rats injected with hamster growth hormone 50 μg/day had significantly increased ($P<0.05$).

TABLE 1. *Bioassay of Growth Hormone of the Hamsters*

Sample	No. of rats	Treated period (days)	Total dose (μg)	Body weight		Tibial cartilage width (μ)
				Initial (g)	Final (g)	
Saline	5	4	-	59.8±2.0[b]	65.6±2.4	175±0
Bovine GH Std.[a]	5	4	100	60.0±1.4	66.6±1.1	252±10***
Bovine GH Std.	5	4	200	60.0±2.1	67.8±3.6	298±18***
Hamster GH	5	4	25	60.0±2.0	67.0±1.2	256±10***
Hamster GH	5	4	50	60.8±2.2	71.6±2.4*	308±40***

a) NIH Standard (Lot No. B-12)
b) Mean+S.D. * $P<0.05$, *** $P<0.001$

TABLE 2. *Bioassay of Prolaction of the Hamsters*

Sample	No. of pigions	Treated period (days)	Total dose (μg)	Activity on the crop sac		
				Pigion number		
				1	2	3
Saline	3	4	-	− −	− −	− −
Hamster PL	3	4	25	+ ±	+ −	Dead
Hamster PL	3	4	100	+ +	+ +	+ +

+ Positive, − Negative

The hamster prolactin was assayed qualitatively by its activity on the crop sac of the pigeon. One group consisting of 3 birds was injected with physiological saline as a control. The other groups were given hamster prolactin 6.25 μg/day and 25 μg/day subcutaneously for 4 days and killed at 24 hr after the last injection. The results of the bilogical assay of prolactin activity are presented in Table 2. The control showed no stimulation of pigeon crop sac. The low dose level of prolactin gave a slight response in the crop sac assay, but the high dose level of prolactin gave a positive response.

Discussion

We have been attempting to identify the constituents of hamster's anterior pituitary gland homogenates seen after electrophoretic resolution on polyacrylamide gel. Four intensely stained components in homogenate of pituitary gland were recognized. The most slowly moving band was identified as growth hormone and the rapidly moving band was identified as prolactin. The other two major bands were identified as albumin and hemoglobin, and we confirmed that the albumin and hemoglobin bands were always more intense when the pituitary galnds were not rinsed before analytical analysis. Lewis et al. (8) have reported that the three major bands seen in electrophoretic patterns of homogenates of mouse pituitary glands were identified as growth hormone and albumin. There was not a band corresponding to prolactin, even though this hormone was seen as a prominent

component in the homogenates of rat pituitary galnds (18). The reason is that prolactin migrates to nearly the same position as albumin in the 7.5 per cent polyacrylamide gel. Cheever et al. (19) have reported that by increasing the concentration of acrylamide to 10 per cent, an excellent resolution of the two proteins was obtained. In the case of hamster, we used the concentration of 7.5 per cent acrylamide and obtained a maximum resolution of all the major components of the electrophoretic pattern.

The isolation procedure of growth hormone and prolactin in the pituitary glands of hamster was at first carried out by the method of Lewis et al. (8). The materials isolated from pituitary glands of hamsters were examined for homogeneity by disk-electrophoresis at pH 9.5. As the isolated materials had many contaminants, we could not obtain sufficient results by this method. Secondly, we tried to isolate the two hormones by cutting out sections of the electrophoretic column following the method of Lewis and Clark. (14). Each section of the column was cut out and homogenized with 0.2 M citrate buffer, pH 5, in a glass homogenizer. The pH of the homogenate was adjusted to 5 with concentrated citric acid. After standing 4 hr at 5°C the homogenate was centrifuged and the supernatant fluid decanted and filtered. The gel was mixed with an equal volume of fresh 0.02 M citrate buffer, pH 5, and allowed to stand 1 hr before centrifuging. The extracts were combined, dialyzed 20 hr against distilled water, and finally lyophilized. As the recovery of protein was so small, this method like the first seemed to be unsuited for the extraction of growth hormone and prolactin from the anterior pituitary of hamsters. At last, we applied an electrophoretical elution technique to the two protein bands within the acrylamide gel columns. Since growth hormone and prolactin were found in fragment readily (20), diisopropylphosphorofluoridate (10^{-3}M) was added to the buffer used for extraction in order to prevent their degradation. We confirmed that prolactin and growth hormone were able to be eluted successfully from the pituitary glands of hamsters. The hamster growth hormone thus obtained had four times the potency of bovine growth hormone (NIH–B12). On the other hand, it is reported by Lewis et al. (21) that the activity of the rat growth hormone isolated by fractionation with ethanol is lower than that of ovine growth hormone (NIH–S5). Also, Jones et al. (18) reported that the activity of the rat growth hormone electrophoretically eluted by the method of Lewis and Clark (14) was compared with ovine growth hormone (NIH–S5) and was found to be equally active. In the present study, however, the activity of the hamster growth hormone was compared with bovine growth hormone (NIH–B12) and the former proved to be four times as active as the latter.

Hamster prolactin is assayed qualitatively by its activity on the crop sac of the pigeon. A high dose level of prolactin gave a positive respose in the crop sac assay, but a low dose level gave a very slight response. It is assumed that

the minimum effective dose of hamster prolactin is 25 μg protein equivalent.

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